Journal of Medical Virology 53:218–224 (1997)

Frequencies of GB Virus C/Hepatitis G Virus Genomes and of Specific Antibodies in German Risk and Non-Risk Populations

C. Micha Nübling,[1]* Heike Bialleck,[2] Andreas J. Fürsch,[3] Inge Scharrer,[4] Wolfgang Schramm,[5] Erhard Seifried,[2] Urban Schmidt,[6] Schlomo Staszewski,[4] and Johannes Löwer[1]

[1]*Paul-Ehrlich-Institut, Langen, Germany*
[2]*Blutspendedienst Hessen des DRK, Frankfurt, Germany*
[3]*Kuratorium für Dialyse und Nierentransplantation, Königstein, Germany*
[4]*Zentrum der Inneren Medizin, Universitätsklinik, Frankfurt, Germany*
[5]*Hämostaseologische Abteilung, Universitätsklinik, München, Germany*
[6]*Boehringer–Mannheim, Penzberg, Germany*

The prevalence of the new flavivirus GB virus C/hepatitis virus G (GBV-C/HGV) in different German populations was investigated by detection of viral genomes and anti-E2 antibodies. While blood donors had an overall prevalence of 10.4% there were increased rates for hemophiliacs (54.7%), hemodialysis patients (30.2%), male homosexuals (30.2%) and intravenous drug users (74.4%). Most GBV-C/HGV positive samples were either viral genome positive or antibody positive, exclusively. Samples with the rare constellation ''positive for both GBV-C/HGV genome and specific antibody'' originated in almost all cases from patients who were additionally infected with HIV or HCV. Probable transmission of GBV-C/HGV by PCR-positive blood transfusions was observed in 5 of 6 cases approximately six months after transfusion. ***J. Med. Virol. 53:218–224, 1997.*** © 1997 Wiley-Liss, Inc.

KEY WORDS: prevalence, GBV-C/HGV-PCR, E2-antibodies, blood donors, transmission

INTRODUCTION

In 1995–1996 a human flavivirus was identified independently by two groups and named GB virus C (GBV-C) [Simons et al., 1996] or hepatitis G virus (HGV) [Linnen et al., 1996]. The term GBV-C/HGV is used in this manuscript since a final decision has not yet been made on the nomenclature of this virus. The GBV-C/HGV genome (10 kb +RNA) has an organisation similar to that of other members of the Flaviviridae family. The new flavivirus is only distantly related to the hepatitis C virus (HCV). There is an overall homology of 27% between the putative polyproteins encoded by the GBV-C/HGV and HCV genomes with maximum homology seen in the NS3 region [Leary et al., 1996]. The two polyproteins are related in molecular size (3,000 aa) and in the order of putative functional domains (structural, non-structural proteins), with the exception that a core encoding segment upstream of the putative env regions (E1, E2) is lacking on the GBV-C/HGV genome [Leary et al., 1996; Muerhoff et al., 1996].

GBV-C/HGV prevalence by PCR analysis has been reported to be in the range between 0.8% and 2% of healthy non-remunerated blood donors [Linnen et al., 1996; Dawson et al., 1996]. The virus may be transmitted by blood transfusion [Linnen et al., 1996; Wang et al., 1996]. A causal relationship between GBV-C/HGV infection and any disease has not been established to date. At least some cases of acute fulminant hepatitis are believed to be associated with GBV-C/HGV [Yoshiba et al., 1995] or not [Kao et al., 1996; Sallie et al., 1996]. A recent report describes some new evidence for an association between a certain GBV-C/HGV strain and fulminant hepatic failure [Heringlake et al., 1996].

Until now, analysis of GBV-C/HGV prevalence in different groups was based on detection of viral genomes by nucleic acid amplification techniques (NAT). The antibody response in GBV-C/HGV-infected individuals seems to be weak and heterogenous although NS3-, NS4- and NS5-specific antibodies have been detected in human sera by Western blot [Pilot-Matias et al., 1996a] and ELISA [Dawson et al., 1996]. With the availability of an ELISA with glycosylated env2-protein (E2) as antigen [Tacke et al., 1997, Dille et al.,

Grant sponsor: "Bundesministerium fur Gesundheit".

*Correspondence to: Dr. C. Micha Nübling, Paul-Ehrlich-Institut, Section of Virology, Paul-Ehrlich-Str. 51-59, 63225 Langen, Germany. E-mail: Nuebling@em.uni-frankfurt.de

Accepted 17 June 1997

1997], the determination of anti-GBV-C/HGV antibodies became more feasible.

Using the polymerase chain reaction (PCR) and the anti-E2 antibody ELISA we investigated the GBV-C/HGV prevalence in several German populations either at risk of viruses transmitted via blood (hemophiliacs, hemodialysis patients, HIV-infected persons) or not (blood donors).

MATERIALS AND METHODS

Plasmas and Sera

Non-risk group: Blood donors. Plasma aliquots of 739 non-remunerated blood donors were provided by "Blutspendedienst Hessen des Deutschen Roten Kreuzes Gmbh, Frankfurt". Blood donors included in this study were recruited from Oberursel and Rüsselsheim which are towns in the Rhein-Main area. Each donation was screened for anti-HIV1/2, anti-HCV, HBsAg and elevated ALT (>45 IU). An additional 100 plasma aliquots with elevated ALT but negative for other screening markers were also included in this study.

Risk groups for viruses transmitted via blood

Hemophiliacs. Sera of hemophiliacs were provided by "Hämostaseologische Abteilung der Uniklinik München" (116 patients) and by "Hämostaseologische Abteilung der Uniklinik Frankfurt" (45 patients). These hemophiliacs have been treated for many years with coagulation factor preparations, manufactured without virus inactivation in the past. This lack of virus inactivation resulted in a positive anti-HCV status in most of these patients (129/161; 80%). Fifty-three of the 129 anti-HCV positive patients were also infected with HIV, giving an overall HIV prevalence of 33% (53/161).

HIV1-/HCV-coinfected intravenous drug users (IVDUs) and HIV-infected male homosexuals. Ninety sera were collected routinely at "Zentrum für Innere Medizin der Uniklinik Frankfurt" from HIV-infected individuals and tested also for anti-HCV. Retrospective analysis of the respective risk groups revealed that all HIV-/HCV-coinfected persons (43) belong to the risk group of intravenous drug users (IVDUs; 17 females, 26 males), while all anti-HCV negative samples (47) originated from male homosexuals.

Hemodialysis patients. Sera from 53 patients on maintenance hemodialysis at the "Kuratorium für Dialyse und Nierentransplantation, Königstein" were selected for their negative status (ELISA, PCR) with regard to HCV, HIV and HBV. Most of the patients had been treated in the past by multiple transfusions of erythrocyte concentrates.

Sample Preparation and GBV-C/HGV PCR

RNA was isolated and purified from 140 μl serum using silica columns provided with the "QIAamp Viral RNA Kit" (QIAGEN, Hilden). The manufacturer's instructions were strictly followed. In the case of blood donor samples, RNA was extracted from minipools consisting of five plasma aliquots each. In this case 2 × 140 μl plasma was lysed and extracted. One-fifth volume (10 μl) from the purified nucleic acids was used for reverse transcription of RNA into cDNA with 50 units cloned M-MLV reverse transcriptase (Applied Biosystems) and random hexamers (2.5 μM). GBV-C/HGV specific nested PCR was carried out with the primers G8 and G9 or G10 and G11 (0.15 μM each; first or second PCR round, respectively) under cycling conditions as described previously [Masuko et al., 1996]. After electrophoresis and staining with ethidium bromide, PCR products were analysed visually. If the PCR result of a blood donor minipool was positive the causative plasma was identified and retested. Each GBV-C/HGV-PCR run was controlled by inclusion of negative and (low-) positive samples. Participation in a GBV-C/HGV-PCR collaborative study organised by E. Schreier (unpublished) and evaluation of our results provides assurance that false-positive or false-negative results were avoided.

Anti-HGV-E2 ELISA

Anti-HGV-E2 antibodies were measured using the research kit "μPlate Anti-HGenv" (kindly provided by Boehringer–Mannheim) which is similar to an enzyme-linked immunosorbent assay (ELISA) described by Tacke et al., [1997]. The test was carried out in accordance with the instructions in the package insert. A complex consisting of biotinylated anti-E2 monoclonal antibody (mouse) and recombinant E2-antigen (expressed in Chinese hamster ovary (CHO) cells) was bound to streptavidin-coated microtiter wells. Human sera or plasma were then incubated; E2-binding human antibodies were detected by POD-conjugate, substrate and analysis of the optical density (OD) values in comparison to the cut-off value. Anti-E2 specificity of human sera was questioned by retesting and parallel performance of the same procedure without E2-antigen (confirmation procedure). Following the instructions in the package insert, anti-E2 reactivity was estimated as confirmed by a maximal reduction factor of 0.66 of the OD in the assay without E2-protein compared to the assay with inclusion of E2-antigen. Intra-assay, inter-assay and inter-lot variation was controlled by regular testing of a dilution series (1:4, 1:16, 1:64) of an anti-E2 reactive sample. The cut-off value (co) was calculated with the help of kit-specific positive (PC) and negative controls (NC) and using the formula: $co = 0{,}2 \times PC + NC$.

Dissociation of putative HGV immune complexes was investigated [Griswold, 1981; von Sydow et al., 1988, modified] by treatment of 50 μl serum or plasma with 50 μl of acid reagens (11% (w/v) glycine, pH 2.0) for 1 hr at 37°C and subsequent neutralisation with 50 μl base reagens (18% (w/v) Tris(hydroxymethyl)-aminomethane, pH 9.0) followed immediately by the anti-E2 ELISA.

TABLE I. Anti-HGV-E2 Specificity of "Grey Zone" Samples (0.5 < sample/cut-off <1.5)

Population	No. grey zone samples/total samples	Anti-HGV-E2 specificity	
		Confirmable	Not confirmable
GBV-C/HGV PCR negative blood donors (ALT < 45 U)	9/196 (4.5%)	2	7
Hemophiliacs	26/161 (16%)	20	6
Hemodialysis patients	7/53 (13%)	5	2
Male homosexuals	2/47 (4%)	2	0
Intravenous drug users	11/43 (26%)	11	0

RESULTS

Interpretation of Borderline Reactivity in the HGV-E2 ELISA

After performance of the HGV-E2 ELISA with samples from low- and high-prevalence groups (see below), it became obvious that samples with OD values near the cut-off value had to be interpreted with caution. Using the cut-off formula given by the manufacturer, a good separation was obtained between non-reactive and reactive samples in low-prevalence populations (blood donors). This distinction was less clear for populations with increased prevalence since quite a large number of sera exhibited elevated OD-values, but would have been classified as non-reactive if the interpretation criteria stated in the package insert had been strictly followed. All samples with sample/cut-off (s/co) ratios in the range between 0.5 and 1.5 (borderline reactivity) were retested in parallel with the confirmation procedure (omission of E2-antigen).

Of the 196 blood donor samples with normal ALT tested by the antibody ELISA, 172 were clearly nonreactive (s/co < 0.3), 15 were anti-E2 reactive (s/co > 1.5) and nine (4.5%) were weakly reactive. In the confirmation test only two of these nine samples were estimated as anti-E2 reactive (Table I). By contrast, sera from populations with increased GBV-C/HGV prevalence had a higher rate of borderline reactivity (in total 46/304; 15.1%) and it was possible to confirm most of the weak-reactive sera (38/46; 82%) as anti-E2 reactive (Table I). Therefore, throughout this study a grey zone (0.5<s/co<1.5) was defined and all grey zone sera were retested with and without E2 antigen. The result of this confirmatory test was decisive.

Blood Donors

In order to determine the rate of GBV-C/HGV viremia in a low-risk population, plasma aliquots from 739 screened blood donors were tested by GBV-C/HGV specific RT-PCR. Seventeen PCR positive plasmas were identified (2.3%). Thirteen of these 17 blood donors agreed to be retested again after eight months. All 13 retested donors were still GBV-C/HGV PCR positive after that period.

Erythrocyte concentrates had been prepared from some of the donations which were found later to be positive for GBV-C/HGV PCR, and had been transfused to recipients. Six of the recipients were tested for potential infection with GBV-C/HGV. Five were PCR positive and anti-E2 negative approximately six months after the transfusion. The investigation of 100 blood donations with increased ALT values gave a PCR positivity rate which was slightly elevated (4/100; 4%) when compared to normal blood donors.

All the 21 PCR-positive (see above) and the 196 PCR-negative plasma aliquots with ALT values in the normal range were also tested for anti-HGV-E2 antibody. All of the 21 PCR-positive plasmas were anti-E2 negative, while 16 of the 196 PCR-negative plasmas (8.1%) were anti-E2 positive (Table II). After dissociation of putative antigen/antibody complexes in the 21 PCR-positive plasmas, the anti-E2 status in these plasmas remained negative. There was also no OD-elevation observed when compared to "non-dissociated" samples indicating absence of anti-E2 in these GBV-C/HGV genome positives. The GBV-C/HGV prevalence among German blood donors based on genome detection plus anti-E2 reactivity is therefore approximately 10% (Table II).

Hemophiliacs

One hundred-sixty one sera of hemophiliacs from two centers (Munich, Frankfurt) were analysed by GBV-C/HGV-PCR and anti-HGV-E2 ELISA. Using these two assays a cumulative prevalence of GBV-C/HGV infections in this population of 54.7% (88/161) was measured. Most of the positive sera were either exclusively anti-E2 reactive (62/161; 38.5%) or exclusively PCR positive (23/161; 14.3%) while only 3 sera (1.9%) were reactive in both GBV-C/HGV-specific assays (Table 2).

The hemophiliac group was subdivided with regard to the HCV and HIV status of the patients. Eighty percent of the hemophiliacs in this study were HCV-positive. All HIV-positive patients (53/161; 33%) were coinfected with HCV. Only 20% of the hemophiliac population in this study was negative for these two virus infections.

In order to measure possible correlations between HCV or HIV and the GBV-C/HGV status, three subgroups (1) HCV-positive/HIV-negative (2) HCV-positive/HIV-positive and (3) HCV-negative/HIV-negative) were analysed with the two GBV-C/HGV assays (PCR, anti-E2 ELISA) (Table III).

The subgroups composed of exclusively HCV positives or of HCV/HIV coinfected persons exhibited a

TABLE II. GBV-C/HGV Prevalence Analysis among Different Populations Determined by GBV-C/HGV-PCR and anti-E2 ELISA

Population	PCR pos anti-E2 neg	PCR neg anti-E2 pos	PCR pos anti-E2 pos	Total prevalence
Blood donors (ALT < 45 U)	17/739 (2.3%)	16/196 (8.1%)	0	10.4%
Blood donors (ALT > 45 U)	4/100 (4%)	ND	0	
161 Hemophiliacs	23 (14.3%)	62 (38.5%)	3 (1.9%)	54.7%
53 Patients on hemodialysis	5 (9.4%)	11 (20.8%)	0	30.2%
47 HIV-pos male homosexuals	4 (8.5%)	9 (19.1%)	2 (4.3%)	31.9%
43 HIV-pos/HCV-pos IVDUs	9 (21%)	18 (41.8%)	5 (11.6%)	74.4%

ND: not done.

TABLE III. Analysis of Hemophiliac Subgroups by GBV-C/HGV-PCR and Anti-HGV-E2

	HCV-positive		HCV-negative
	HIV-negative (n = 74)	HIV-positive (n = 53)	HIV-negative (n = 32)
GBV-C/HGV PCR-pos	12 (16.2%)	12 (22.6%)	2 (6.2%)
Anti-HGV-E2 pos	35 (47%)	20 (37.7%)	10 (31%)
Prevalence: GBV-C/HGV PCR-pos and/or anti-HGV-E2 pos	45 (60.8%)	32 (60.3%)	11 (34%)

very similar GBV-C/HGV total prevalence of about 60%. The highest rates of PCR-positive and presumably viremic sera were determined in the same two subgroups with 22.6% and 16.2% for the groups with and without HIV-markers, respectively.

By contrast, the hemophiliac subgroup without HCV or HIV markers exhibited a total GBV-C/HGV prevalence of 34%, and 6.2% of the members of this group were GBV-C/HGV PCR-positive.

Hemodialysis Patients and HIV-Infected Persons

Most of the 53 hemodialysis patients had a well-documented history of multiple transfusions. The members of this group had been preselected for negative markers for HIV, HCV, and HBV. Despite preselection, 5 GBV-C/HGV viremic and 11 E2antibody-reactive individuals were identified. There was no individual positive for both markers. These results account for a total GBV-C/HGV prevalence of 30.2% among hemodialysis patients (Table 2).

A group of 90 HIV-positive persons attending an infection clinic was subdivided retrospectively for the respective risk of their HIV-infections. This analysis revealed that two main risks were associated with HIV-infection. The first subgroup consisted of 43 intravenous drug users (IVDUs) who were all coinfected with HCV, while the second subgroup consisted of 47 HCV-negative male homosexuals.

In this study the highest GBV-C/HGV prevalence (74.4%) was determined for HIV-positive IVDUs, of whom 32.6% were PCR-positive and 53.4% anti-E2 reactive. Among them 5 persons (11.6%) were positive for both markers (Table 2). The GBV-C/HGV prevalence of HIV-positive male homosexuals was 31.9% which is composed of 12.8% PCR-positives and 23.4% anti-E2 reactives with 4.3% being positive for both markers.

DISCUSSION

In this study, we investigated the frequencies of virus genome detection and antibody detection caused by infections with the human flavivirus GBV-C/HGV. Both markers account for the total prevalence rate of a population. Most GBV-C/HGV infected individuals were either PCR-positive or anti-GBV-C/HGV reactive.

From 179 GBV-C/HGV-infected individuals (127 with and 55 without HCV- or HIV-coinfections) identified in the different populations, only 10 (5.5%) were both PCR- and anti-E2 positive. Nine of these 10 individuals were coinfected with either HIV plus HCV (5), HIV only (2) or HCV only (2).

These data suggest, firstly, a direct correlation between antibody appearance and putative GBV-C/HGV clearance through immune mechanisms. This is in strong contrast to the course of most HCV infections [Alter, 1996]. Secondly, coinfection with HIV and/or HCV may weaken this putative antibody-mediated clearance of GBV-C/HGV infections since nine of the ten individuals with the rare constellation PCR-positive + anti-E2 positive were HIV- and/or HCV-infected. The diverse immune-suppressive effects of HIV infection might explain the failure for clearance of GBV-C/HGV despite the presence of specific antibodies.

A significant proportion of GBV-C/HGV infected persons seems to remain persistently infected over long periods, since up to approximately 30% of GBV-C infections may remain anti-E2 negative and PCR-positive over a number of years as shown by a corresponding analysis of several follow-ups (data not shown; [Pilot-Matias et al., 1996b]). In all populations investigated, the number of anti-E2 reactive sera exceeds the number of viral RNA-positive sera by the factor 2 - 4, irrespective of the specific prevalence in the population. This again is in strong contrast to HCV where by far most (if not all) anti-HCV negative, HCV-PCR positive sera represent the very early phase (diagnostic window) of an infection.

Until recently, GBV-C/HGV prevalence studies were based solely on the detection of viral genomes by nucleic acid amplification techniques (NAT). By eukaryotic expression of glycosylated GBV-C/HGV envelope protein (E2), diagnostic companies have now succeeded in developing research ELISAs [Dille et al., 1997; Tacke et al., 1997] or a RIPA [Pilotmatias et al., 1996] suitable for investigating populations. Several investigators described difficulties in the determination of humoral anti-GBV-C/HGV responses. Possible reasons are the relatively weak anti-GBV-C/HGV response or the chosen expression system for recombinant antigens. Prokaryotic expression may have the intrinsic disadvantage of different or of lacking post-translational modifications (e.g. glycosylation) which are potentially critical for representing natural epitopes.

Since there is evidence for a variety of different antibody targets in anti-GBV-C/HGV positive sera [Dawson et al., 1996; Pilot-Matias et al., 1996a] recombinant E2 might represent an antigen which is recognized by only a subset of anti-GBV-C/HGV positive persons. Therefore for the time being an underestimation of antiGBV-C/HGV positive sera can not be totally excluded completely by using this single antigen test.

We also suggest retesting all weakly reactive samples with a confirmatory assay since (depending on the prevalence situation) false-negative or false-positive results caused by borderline sera may give inaccurate results.

For German blood donors we determined a total GBV-C/HGV prevalence (PCR plus antibodies) of 10%. In this group no case with coexistence of genomes and E2-specific antibodies was detected. Since E2 is a component of the putative viral particle specific antibodies could be hidden in immune-complexes present in PCR-positive samples. Dissociation of the putative aggregates by acid treatment and testing for anti-E2 revealed no evidence for this hypothesis. In contrast, for HIV-infected individuals dissociation of anti-p24 and p24 was proven to increase OD in anti-p24 ELISA as well as in p24 antigen assays (data not shown). A low anti-E2 level undetectable in this test system cannot be excludud definitely.

Published NAT data on blood donors are largely consistent with our PCR results. Non-remunerated blood donors in the USA and in Europe have been found PCR-positive in the range from 0.8% [Dawson et al., 1996] to 1.7% [Linnen et al., 1996]. Roth and colleagues [Roth et al., 1997] determined a higher PCR rate in German donors living in towns or cities (2.2%) compared with donors from small villages in rural areas (0.8%). The blood donors included in our study were recruited from two German towns, and the 2.3% PCR-positivity therefore agrees very closely with the data of Roth and colleagues.

Published GBV-C/HGV PCR data on blood donors with elevated ALT and their interpretation are contradictory, showing a higher GBV-C/HGV prevalence in this population [Dawson et al., 1996; Roth et al., 1997] or not [Linnen et al., 1996]. We found a slightly higher PCR rate (4%) in 100 donors with elevated ALT-values. Nevertheless, investigation of a much larger number of blood donors with elevated ALT is necessary in order to determine the real significance.

The follow-up studies of the GBV-C/HGV PCR positive donors confirmed the viremic state of these individuals after 8 months without an indication of any disease. These donors therefore continue to be classified among asymptomatic chronic carriers of GBV-C/HGV (persistently infected persons). Transmission of GBV-C/HGV by respective erythrocyte concentrates is the most probable explanation for the PCR positivity of most of the recipients, and GBV-C/HGV PCR-positive transfusions must therefore be considered as potentially infectious. Recipients had not been PCR tested at the time of transfusion, and a positive status before transfusion therefore cannot be definitely ruled out. Bearing in mind the PCR prevalence of GBV-C/HGV in non-risk groups, any other cause for these infections in recipients would seem to be highly unlikely. The investigation of further follow-up samples from these recipients will clarify the rate of anti-E2 seroconversion among them.

A recent publication [Jarvis et al., 1996] raised the obvious discrepancy between the infection rates for HCV (83%) and GBV-C/HGV (14%) among a Scottish hemophiliac population, which has a composition that is very similar to the corresponding group in our study where 80% of the hemophiliacs are HCV-infected and only 16% positive in GBV-C/HGV-PCR. The published observations (see above) were based exclusively on GBV-C/HGV-PCR data. After inclusion of our anti-E2 data an unexpected discrepancy between HCV-prevalence (80%) and GBV-C/HGV-prevalence (54.7%) still exists also in our hemophiliac population, but is greatly reduced. PCR investigations of clotting factor concentrates show that, before the introduction of virus inactivation measures, many batches were GBV-C/HGV positive whereas HCV is detected less frequently in these products; this is in accordance with the relative prevalence of these viruses in the blood donor population. After the introduction of virus inactivation procedures, viral genomes are still, detected but less frequently, in some factor concentrates [Jarvis et al.,

1996; Garciatrevijano et al., 1996] or other blood derivatives [Nübling and Löwer, 1996].

The apparent lower infection rates of GBV-C/HGV versus HCV in hemophiliacs may be explained by possible underestimations of GBV-C/HGV-infections because of suboptimal diagnostics or by the disappearance of anti-GBV-C/HGV antibodies after virus clearance. Also the cause might be a lower infectivity of GBV-C/HGV compared with HCV. Furthermore, the presence of anti-HGV antibodies with virus neutralisation capacity in earlier coagulation factor concentrates could have had an impact on the lower transmission rate. Studies on follow-ups of fresh infections are necessary for resolving these questions.

A GBV-C/HGV-prevalence of 30.2% was found in hemodialysis patients. The PCR-prevalence of 9.4% (5/53) is higher than the corresponding 3.1% determined for Japanese patients [Masuko et al., 1996] and lower than the rate of 19% in Italian hemodialysis patients [Sampietro et al., 1996]. Preselection of our patients in terms of a negative status for other viral markers as well as different patient histories may explain these differences.

Four of our five PCR-positive hemodialysis patients had a well-documented history of multiple transfusions. This treatment combined with the relatively high rate of GBV-C/HGV viremia in blood donors, or the threat of nosocomial infections generally associated with maintenance hemodialysis is the probable explanation for the increased GBV-C/HGV-prevalence in this group.

In our study, HIV-positive IVDUs were the group with the highest GBV-C/HGV-prevalence (74.4%). This group was preselected with regard to the risk of viruses transmitted via blood, and a variety of infection markers among IVDUs is well-known. Since most members of this group are still intravenous drug users, there is a permanent risk of boostering or reinfections. Together with the immune-suppressive effect of HIV-coinfection, this might explain the high prevalence and the high rate of viremic individuals (32.6%).

The prevalence of 31.9% found in HIV-infected male homosexuals suggests a transmission mechanism of GBV-C/HGV which is distinct from HCV. HCV is not (or very rarely) transmitted by sexual intercourse and is not prevalent in this group. Analysis of HIV-negative homosexuals is necessary to decide if coinfection with HIV has an impact on the increased prevalence rate.

Prevalence studies for GBV-C/HGV must include NAT as well as sensitive antibody tests since GBV-C/HGV infections may take two main courses (persistent infections with long-lasting viremias or virus clearing after antibody development).

A combination of methods for prevalence measurements as well as further studies on the biology of this virus are necessary for forming an accurate impression of the clinical significance and the complete course of GBV-C/HGV infections.

ACKNOWLEDGMENTS

The authors thank Ines Amberg and Nicolle Münzer for excellent technical assistance, Ina Plumbaum and Dr. Steve Norley for critically reading the manuscript. This study was supported by a grant from "Bundesministerium für Gesundheit".

REFERENCES

Alter HJ (1996): The cloning and clinical implications of HGV and HGBV-C. New England Journal of Medicine 334:1536–1537.

Dawson GJ, Schlauder GG, Pilotmatias TJ, Thiele D, Leary TP, Murphy P, Rosenblatt JE, Simons JN, Martinson FEA, Gutierrez RA, Lentino JR, Pachucki C, Muerhoff AS, Widell A, Tegtmeier G, Desai S, Mushahwar IK (1996): Prevalence studies of GB virus-C infection using reverse transcriptase polymerase chain reaction. Journal of Medical Virology 50:97–103.

Dille BJ, Surowy TK, Gutierrez RA, Coleman PF, Knigge MF, Carrick RJ, Aach RD, Hollinger FB, Stevens CE, Barbosa LH, Nemo GJ, Mosley JW, Dawson GJ, Mushahwar IK (1997): An ELISA for detection of antibodies to the E2 protein of GB virus C.Journal of Infectious Diseases 175:458–461.

Garciatrevijano ER, Lopezalcorocho JM, Quintana M, Hernandez F, Carreno V (1996): HGV in coagulation-factor concentrates. The Lancet 348:1032.

Griswold WR (1981): A modified Farr technique for the detection of antibody in the presence of antigen-antibody complexes. Journal of Immunological Methods 40:73–78.

Heringlake S, Osterkamp S, Trautwein C, Tillmann HL, Boker K, Muerhoff S, Mushahwar IK, Hunsmann G, Manns MP (1996): Association between fulminant hepatic failure and a strain of GBV virus C. The Lancet 348:1626–1629.

Jarvis LM, Davidson F, Hanley JP, Yap PL, Ludlam CA, Simmonds P (1996): Infection with hepatitis G virus among recipients of plasma products. The Lancet 348:1352–1355.

Kao J-H, Chen P-J, Chen D-S (1996): GBV-C in the aetiology of fulminant hapatitis. The Lancet 347:120.

Leary TP, Muerhoff AS, Simons JN, Pilot-Matias TJ, Erker JC, Chalmers ML, Schlauder GG, Dawson GJ, Desai SM, Mushahwar IK (1996): Sequence and genomic organization of GBV-C: A novel member of the flaviviridae associated with human non-A-E hepatitis. Journal of Medical Virology 48:60–67.

Linnen J, Wages J, Zhangkeck ZY, Fry KE, Krawczynski KZ, Alter H, Koonin E, Gallagher M, Alter M, Hadziyannis S, Karayiannis P, Fung K, Nakatsuji Y, Shih JWK, Young L, Piatak M, Hoover C, Fernandez J, Chen S, Zou JC, Morris T, Hyams KC, Ismay S, Lifson JD, Hess G, Foung SKH, Thomas H, Bradley D, Margolis H, Kim JP (1996): Molecular cloning and disease association of hepatitis G virus: A transfusion-transmissible agent. Science 271:505–508.

Masuko K, Mitsui T, Iwano K, Yamazaki C, Okuda K, Meguro T, Murayama N, Inoue T, Tsuda F, Okamoto H, Miyakawa Y, Mayumi M (1996): Infection with hepatitis GB virus C in patients on maintenance hemodialysis. New England Journal of Medicine 334:1485–1490.

Muerhoff AS, Simons JN, Leary TP, Erker JC, Chalmers ML, Pilotmatias TJ, Dawson GJ, Desai SM, Mushahwar IK (1996): Sequence heterogeneity within the 5′-terminal region of the hepatitis GB virus C genome and evidence for genotypes. Journal of Hepatology 25:379–384.

Nübling CM and Löwer J (1996): GB-C genomes in a high-risk group, in plasma pools, and in intravenous immunoglobulin. The Lancet 347:68.

Pilot-Matias TJ, Muerhoff AS, Simons JN, Leary TP, Buijk SL, Chalmers ML, Erker JC, Dawson GJ, Desai SM, Mushawar IK (1996a): Identification of antigenic regions in the GB hepatitis viruses GBV-A, GBV-B, and GBV-C. Journal of Medical Virology 48:329–338.

Pilot-Matias TJ, Carrick RJ, Coleman PF, Leary TP, Surowy TK, Simons JN, Muerhoff AS, Buijk SL, Chalmers ML, Dawson GJ, Desai SM, Mushahwar IK (1996b): Expression of the GB virus C E2 glycoprotein using the Semliki Forest virus vector system and its utility as a serologic marker. Virology 225:282–292.

Roth WK, Waschk D, Marx S, Tschauder S, Zeuzem S, Bialleck H,

Weber H, Seifried E (1997): Prevalence of hepatitis G virus and its strain variant, the GB agent, in blood donations and their transmission to recipients. Transfusion 37:651–656.

Sallie R, Shaw J, Mutimer D (1996): GBV-C virus and fulminant hepatic failure. The Lancet 347:1552.

Sampietro M, Badalamenti S, Lunghi G (1996): Hepatitis GB virus C. New England Journal of Medicine 335:1392.

Simons JN, Leary TP, Dawson GJ, Pilot-Matias TJ, Muerhoff AS, Schlauder GG, Desai SM, Mushawar IK (1996): Isolation of novel virus-like sequences associated with human hepatitis. Nature Medicine 1:564–569.

Tacke M, Kiyosawa K, Stark K, Schlueter V, Ofenloch-Haehnle B, Hess G, Engel AM (1997): Detection of antibodies to a putative hepatitis G virus envelope protein. The Lancet 349:318–320.

von Sydow M, Gaines M, Sonnerborg A, Forsgren M, Pehrson PO, Strannegard O (1988). Antigen detection in primary HIV infection. British Medical Journal 296:238–240.

Wang JT, Tsai FC, Lee CZ, Chen PJ, Sheu JC, Wang TH, Chen DS (1996): A prospective study of transfusion-transmitted GB virus C infection: Similar frequency but different clinical presentation compared with hepatitis C virus. Blood 88:1881–1886.

Yoshiba M, Okamoto H, Mishiro S (1995): Detection of the GBV-C hepatitis virus genome in serum from patients with fulminant hepatitis of unknown aetiology. The Lancet 346:1131–1132.